REMARKS

I. Status of the claims and application

Claims 1-10 and 12-18 are pending. Claim 2 has been cancelled without prejudice or disclaimer. Claims 1, 7, and 8, have been amended.

II. Summary of the Invention

The present invention envisions a "microspore-specific" promoter and a method for producing hybrid plants with "gametophytic male sterility." The invention uses "a promoter region which controls the expression, specifically in the microspores, of a gene encoding a cytotoxic molecule." Specifically, the invention employs the promoter region of the nucleotide sequence denoted as SEQ ID NO. 3, *i.e.*, nucleotides 1 to 2056 to drive expression of another nucleic acid "specifically in the microspore." See page 3, lines 14-18. The promoter region of SEQ ID NO. 3 "is defined as being the sequence preceding (on the 5' side [of]) the translation start codon." See page 3, lines 28-31.

A vector construct of the present invention, therefore, comprises **the promoter region of SEQ ID NO. 3** fused to "another [open reading frame] whose product is a cytotoxic molecule [which is] capable of destroying" microspores. See page 4, lines 4-10, and page 6, lines 27-31. The toxicity of such a molecule can also be controlled according to the present invention. See page 2 of the specification at lines 33-37 and, similarly, page 3, lines 1-2. For instance, the cytotoxic effects of a "subtilisin," when fused to the promoter region of SEQ ID NO. 3, can be inhibited by applying flurophosphate insecticide to the plant. See page 7, lines 7-13.

III. The Office Action

The Examiner has maintained his rejections of claims 1-10 and 12-18 under Section 112, paragraph 1; claim 7, under Section 112, paragraph 2; claims 1-3, 5-9, and 13-14 under Section 102(e); claims 1-3, 5-9, and 12-17 under Section 102(e); and claim 4 under Section 103(a).

IV. Overcoming the Examiner's Rejections

(i) Claims 1-10 and 12-18 are enabled

(a) It is well within the purview of the skilled artisan to identify sequence variants and fragments of SEQ ID NO. 3 that are functional promoters

The Examiner acknowledges that claims drawn to bases 1 to 2056 of SEQ ID NO. 3 satisfy the Written Description requirement, but alleges that "claims drawn to anything less than the full sequence does not adequately describe Applicants' invention," Office Action at page 3. According to the Examiner, "without specifically identifying and describing the functional domains of the promoter, one skilled in the art would not be able to identify sequences exhibiting 80% sequence identity or fragments of SEQ ID NO. 3 that still possessed the necessary domains to direct expression of a heterologous gene with the same spatial and temporal expression as bases 1 to 2056 of SEQ ID NO. 3," Office Action at page 3.

To the contrary, the skilled artisan can access a wealth of structural and functional information describing specific features and characteristics of eukaryotic promoters and the domains involved in gene expression. The Examiner is directed to Amgen Inc., Plaintiff-Cross Appellant, v. Hoechst Marion Roussel, Inc. (now known As Aventis Pharmaceuticals Inc.) and Transkaryotic Therapies, Inc., Defendants-Appellants. Court Of Appeals For The Federal Circuit, 314 F.3d 1313; 2003 U.S. App. LEXIS 118; 65 U.S.P.Q.2D (BNA) 1385. There, the Court held that the Written Description "focuses on a comparison between the specification and the invention referenced by the terms of the claim," and that the requirement "may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure." *See*, pages 9 and 10.

Accordingly, it is well established in the art that certain transcription factors bind to specific sequences present in eukaryotic promoters. *See*, Werner T., "*Models for prediction and recognition of eukaryotic promoters*," Mammalian Genome, 10, 168-175, 1999, which is appended to this Amendment. At page 169, Werner describes the "basic structure of a polymerase II promoter," *i.e.*, the eukaryotic polymerase involved in gene transcription. Such a promoter, he informs, may comprise a "transcription start site," an "initiator region," "transcription factor binding sites," and "one or several essential binding sites [such as the "TATA" box] for general transcription factors."

According to Werner, "the region of the promoter that is sufficient to determine the precise transcription start site" is the "core promoter." Werner also analyzes in great detail, the state of various promoter "prediction" programs and their ability to define particular regions, sequences, and domains that characterize a "promoter." Werner assumed, in 1999, that "the general promoter prediction is already about as good as it gets, and I do not expect more than moderate increases in specificities." He concluded that "I cannot see any alternative to computer-assisted evaluation of genomic sequences prior to experimental analysis." Accordingly, there exists numerous mechanisms, in addition to a wealth of literature, from which the skilled artisan can retrieve basic and detailed information for determining which portions or fragments of SEQ ID NO. 3 are likely to be involved in transcription of a gene. This is especially more so, given that Applicants have shown that bases 1 to 2056 of SEQ ID NO. 3 function in such a manner.

Furthermore, Applicants have disclosed that SEQ ID NO. 3 is a male gametophyte specific promoter, and that this promoter "contains three dispersed copies of a motif described previously in the promoters of several genes expressed in the male gametophyte, Fourgoux-Nicol *et al.*, Plant Molecular Biology, 40(5), pp.857-72, 1999 (appended to this Amendment). There, Applicants characterize the same BnM3.4 clone that they described in the present application. See, for instance, page 867, "*The BnM3.4 promoter: regions of homology and functional analysis*."

Moreover, it is now routine practice, and certainly not undue experimentation, to create "promoter deletion constructs" operably linked to a reporter gene, such as luciferase, in order to determine which regions or fragments of a promoter are required for transcription.

Thus, it is well within the purview of the skilled artisan to "identify" sequences that are important for transcription and, therefore, well within their ability to determine sequences that have 80% sequence identity with SEQ ID NO. 3 or a part thereof, yet are still functional promoters.

Nevertheless, for the sole reason of expediting prosecution, Applicants have amended the claims to recite a purified polynucleotide consisting essentially of nucleotides 1 to 2056 of SEQ ID NO. 3, which is capable of expressing a second polynucleotide. According to the Examiner's own rationale, that "the disclosure of bases

1 to 2056 satisfies the Written Description requirement," Applicants respectfully request that the Examiner withdraw this rejection.

(b) <u>There is no requirement for Applicants to recite the genotype or phenotype of a claimed plant in order to establish patentability</u>

The Examiner states that, for the purposes of satisfying the Written Description, "Applicant is required to disclose the genotype and phenotype of plants that Applicant is claiming," Office Action at page 3. "Applicant is claiming plant material but Applicant hasn't disclosed the genotype of the claimed plant."

Applicants respectfully contend that the Written Description requirement does not call for the recitation of the genetic makeup (*i.e.*, the "genotype") or the physical attributes (*i.e.*, the "phenotype") of the claimed plants in the pertinent claims. The present specification asserts that "the present invention thus enables the production of plants with gametophytic male sterility which inhibits any production of pollen grains," page 5, lines 19-22. Furthermore, Applicants describe at pages 5-8 the various haploid, diploid, heterozygous, homozygous, and hemizygous states of plants that are transformed and manipulated according to the present invention. Indeed, when such plants, *i.e.*, those that are male gametophyte sterile, are cultivated without a fertility inductor, 50% of the resultant pollen are viable. Thse viable pollen being precisely those which do not carry the transgene.

The plant of claim 16 is limited to the *Brassicaceae* family of plants. Furthermore, Applicants exemplify the *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the inventive gamete-specific promoter construct, as one particular example of how the inventive promoter may be used. The results of Example 1, part B, at pages 12 and 13 of the specification show that β-glucuronidase, operably linked to the promoter of SEQ ID NO. 3, was specifically expressed in the microspores of *Arabidopsis thaliana*.

However, the present invention is not limited to the transformation of only *Brassicaceae* or *Arabidopsis thaliana* plant species. The inventive promoter of SEQ ID NO. 3 can be introduced into *any* plant: "[T]he transformation of other plants, and in particular rape, may be carried out" through *Agrobacterium*-mediated transformation (specification at page 4). Thus, claims 6 and 7 are simply directed to "plants" whose cells comprise a vector that contains the inventive promoter sequence of SEQ ID NO. 3

(claim 6), and to plants with gametophytic male sterility that comprise a gene encoding a male gamete-specific cytotoxic product.

Accordingly, there is adequate written description for plants comprising a vector that contains the inventive promoter sequence operably linked to a polynucleotide that encodes a cytotoxic product. It is unnecessary for Applicants to recite "the genotype of the claimed plant" in the claims. The genotype of the claimed plant is irrelevant to the claimed subject matter. Therefore, Applicants respectfully request that the Examiner withdraw this rejection.

(c) **Elucidating "how" the presently claimed invention works is not a necessary requirement for establishing patentability**

The Examiner alleges that "it is unclear ***how*** expressing a protein which is already present in the microsporocytes to begin with will have an adverse effect on the developmental biology of the microsporocyte," (emphasis added), Office Action at page 4. "Therefore, given the lack of experimental data which demonstrates that expressing subtilisin in the microspores will create male sterile pollen, the Examiner maintains the assertion that the invention is not enabled, because subtilisin is already present in the microspores."

Applicants disagree with the Examiner's generalization. The present claims require the promoter of SEQ ID NO. 3 to be operably linked to a polynucleotide that encodes a "cytotoxic" product. The claims also recite that that expression product is "cytotoxic to a microspore." Furthermore, the cytotoxic product can be subtilisin. Whether or not the expression product "is already present" in a microspore, as the Examiner questions, is irrelevant to the claimed subject matter. The claims do not require a microspore to be deficient in a protein that is subsequently expressed using the inventive construct and promoter, nor do they require that the promoter of SEQ ID NO. 3 be operably linked to a gene encoding an endogenous protein. It is an object of the present invention to "control the expression, specifically in the microspores, of a gene encoding a cytotoxic molecule," specification at page 2, lines 35-38.

It is not necessary to explain *how* the cytotoxic product promotes an "adverse effect" on a microspore, nor is it necessary to elucidate the mechanism by which a protein, such as a protease, destroys proteins so that the "microspore cannot survive" (specification at page 4, lines 15-19).

Furthermore, the basis for the Examiner's non-enablement rejection lies in his sweeping generalization that "subtilisin is already present in the microspores," yet he provides no examples, nor does he reference specific plants or plant species to support this assertion. Moreover, the present invention is not directed solely to the expression of a subtilisin cytotoxic product in microspores, but encompasses any cytotoxic product whose expression destroys the microspore in which it is expressed. Accordingly, the rejection of the present claims as non-enabled simply because the application does not disclose a working example of one species of cytotoxic product, is improper.

Indeed, "lack of experimental data," is not sufficient grounds for rejecting Applicants' claimed invention. "In other words, lack of working examples or lack of evidence that the claimed invention works as described should never be the sole reason for rejecting the claimed invention on the grounds of lack of enablement," MPEP, Section 2164.02. The Examiner, however, regards the absence of an example demonstrating "that expressing subtilisin in the microspores will create male sterile pollen," as the reason for rejecting the claims: "*Therefore*, given the lack of experimental data [to this effect] . . . the invention is not enabled" (emphasis added).

In actual fact, there is no plant "subtilisin" protein *per se*, only plant "subtilisin-like" proteases. Thus, the Examiner's rejection is, from the start, flawed. He further assumes that all plants and all plant microspores naturally express a bacterial subtilisin protein. Even if all plants and all plant microspores did somehow naturally express a *bona fide* bacterial "subtilisin" protein, that fact would not alter the patentability of the claimed subject matter. Likewise, the present claims do not require "expressing a protein which is already present in the microsporocytes" as alleged by the Examiner. Claims 3, 4, 8, 9, and 18, simply require that the promoter of the present invention is operably linked to another polynucleotide that encodes for a "cytotoxic product." Accordingly, Applicants respectfully request that the Examiner withdraw this rejection.

(d) **Applicants do not have to demonstrate, experimentally, that an insecticide can be taken up through a cuticular wax membrane to establish patentability**

The Examiner dismisses Applicants inventive step for inducing male fertility by applying an insecticide to the transformed plant, because "given the problems of chemical absorption through cuticular waxes, Applicant has not taught or presented

examples which disclose how one skilled in the art is to achieve the desired effect without undue experimentation," Office Action at page 5.

Again, the Examiner has rejected the present claims because they have not, according to the Examiner, presented a solution to "problems" associated with "chemical absorption through cuticular waxes" in order to practice the claimed invention. And again, the Examiner seeks confirmation of patentability from Applicants by the presentation of working examples that show uptake of a chemical through the cuticular wax of a plant. Furthermore, the Examiner has failed to provide Applicants with a specific instance or publication to support his position.

The scientific literature is replete with information regarding the species-to-species differences in plant cuticular composition and permeability. Indeed, studies dating back from the early Eighties show accumulation and transport of herbicides, such as (2,4-dichlorophenoxy acetic acid), as an indicator of cuticular membrane permeability (Riederer & Schonherr, Ecotoxicol Environ Saf, 9(2), p.168, 1985, abstract appended), and the uptake of phenol, 2-nitrophenol, and 4-nitrophenol in plant cuticles (Shafer & Schonherr, Ecotoxicol Environ Saf, 10(2), p.239, 1985, abstract appended). Studies also show that aqueous pores in plant cuticles facilitate penetration of chemicals, such as calcium chloride; that surfactants can affect the penetration of compounds through the cuticular; and that cuticular permeability is dependent upon humidity and temperature. Accordingly, the art is clear on the parameters and features of certain plant cuticular membranes that affect permeability.

Applicants infer that the Examiner has interpreted the step of "applying" an flurophosphate molecule "to a plant," as recited in present claim 10, to mean applying the insecticide molecule only to the leaves, *i.e.*, the cuticular membranes, of the plant. However, Applicants clearly state in the application that the insecticide "may be applied to the foot of the plant," *i.e.*, to the base of the plant, whereupon the insecticide is taken up into the plant tissue via the roots of the plant. See, page 7, lines 13-17, of the present specification. Such "systemic insecticides" are well known to the skilled artisan for conferring poisonous chemicals to the sap of plants that are hosts for certain insects. It also is well known that solutions of such systemic insecticides can be injected directly into the ground, or soil, near the plant.

Accordingly, the Examiner has no basis for rejecting the claims simply because there is no example in the application demonstrating the uptake of an insecticide through a plant cuticular waxy membrane. The Examiner's rejection is improper and Applicants respectfully request that the Examiner withdraw this rejection.

(ii) Claim 7 is free from objection

The Examiner rejected claim 7 under 35 U.S.C. § 112, second paragraph, because it is directed to a "gene encoding a male-gamete-specific cytotoxic product" and, therefore, according to the Examiner "still embraces subject matter not intended by Applicant," Office Action at page 6. The Examiner suggested that Applicants delete "male-gamete specific" before "cytotoxic" and insert instead "operably linked to a male-gamete-specific promoter." Applicants thank the Examiner for this recommendation, and have duly amended claim 7. According to the Examiner's own rationale, therefore, this amendment "obviates this rejection" and Applicants respectfully request that the Examiner, therefore, withdraw this rejection.

(iii) The claims are not anticipated or rendered obvious by the prior art

The Examiner states that "Applicants do not claim a gametophytic promoter; rather they claim a promoter that expresses in a gene [sic] that produces a cytotoxic product that produces male sterile plants," Office Action at page 7.

The Examiner's understanding of the present invention is misguided. Applicants' do in fact claim a promoter whose expression is directed specifically to the gametes of a plant. Applicants show in Example 2 at page 11 of the application, that the promoter comprising the sequence of SEQ ID NO. 3, as captured in clone BnM3.4, is functional only in the microspores. "No coloration is present in the adjacent tissues of the anther, nor in the somatic tissues of the plant. In a transformed plant which is hemizygous for the chimeric gene, half the microspores produced are blue [due to expression of β-glucuronidase], because only they contain the chimeric gene" driven by the inventive gametophyte-specific promoter. Applicants have amended the claims to clarify and make explicit that their inventive promoter is, indeed, a "gametophytic promoter."

The Examiner inexplicably maintains that a single nucleotide base is a comprehensible "fragment" of SEQ ID NO. 3, and that that single nucleotide is "capable

of expressing a second nucleotide sequence to which it is operably linked," as prescribed by the present claimed invention.

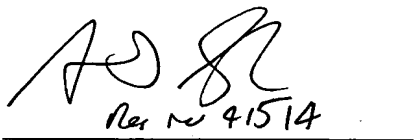
Claim 1 of the present invention is directed to a purified nucleotide sequence that comprises a "nucleotide sequence which is a fragment" of residues 1-2056 of SEQ ID NO. 3, or a fragment of a nucleotide sequence that has at least 80% homology with residues 1-2056 of SEQ ID NO. 3. The claim requires that the purified nucleotide sequence must be "capable of expressing a second nucleotide sequence to which it is operably linked." Applicants have amended the claim to make explicit that the nucleotide sequences of "a)," "b)," and "c)," are also capable of expressing a second nucleotide sequence to which it is operably linked." Accordingly, a "fragment" of claim 1 must be a gametophyte-specific promoter, and since a single nucleotide is not a gametophyte-specific promoter, nor is a single nucleotide capable of expressing a cojoined polynucleotide, then claim 1 does not read on any of the cited prior art "promoters."

Since the claims have been so amended, Applicants also successfully traverse the Examiner's rejection of the claims as obvious over Mariani *et al.* in light of Ramjee *et al.* Accordingly, none of the claims are anticipated or rendered obvious by the cited prior art, and Applicants respectfully request that the Examiner withdraw these rejections.

IV. Conclusion

In view of the foregoing, applicants submit that the present claims are free from objection and earnestly solicit an early notice of allowance. Nevertheless, should there be any questions, Examiner Baum is courteously invited to contact the undersigned attorney at the telephone number shown below.

Respectfully submitted,



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MARKED-UP VERSION OF THE CLAIMS

1. (Twice amended) A purified nucleotide sequence **consisting essentially of** [comprising]:

a) **nucleotides 1-2056 of** [at least a part of] SEQ ID NO. 3, or

b) a sequence which has at least 80% homology with a), **or**

c) a fragment of the nucleotide of a) or b)

wherein said purified nucleotide sequence is capable of expressing a second nucleotide sequence to which it is operably linked**, and wherein said purified nucleotide sequence is a gametophytic-specific promoter and wherein the nucleotide sequences of a), b) and c) are all capable of expressing a second nucleotide sequence to which they are operably linked.**

7. (Amended) A plant having gametophytic male sterility with inducible fertility, comprising a gene encoding a [male-gamete-specific] cytotoxic product **which is operably linked to a male-gamete-specific promoter**.

8. (Amended) A method for producing a plant with gametophytic male sterility with inducible fertility, comprising inserting into one or more plant cells a gene **that is operably linked to a gametophyte-specific promoter** [present in a construct], wherein the expression product of said gene is cytotoxic to a microspore; and producing a plant therefrom which does not produce a male gamete**, wherein said gametophyte-specific promoter consists essentially of nucleotides 1-2056 of SEQ ID NO. 3, or fragment thereof, wherein said promoter and said fragment are capable of expressing said gene**.